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Physiologically Active Agents containing Derivatives of (3-dimethylcarbamoxyphenyl) tri-methyl ammonium

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DETAILS

1. Title of this Invention

Physiologically Active Agents containing Derivatives of (3-dimethylcarbamoxyphenyl) tri-methyl ammonium

2. Claim

(1) Physiologically active agents which have activities of antitumor, reducing sugar and lipid levels in blood, anti-inflammatory, antipyretic and sedative, containing derivatives of (3-dimethylcarbamoxyphenyl) trimethyl ammonium as an active component shown in a chemical formula in which R represents methyl sulfate radical (CH_3SO_4-) or bromine atom.

(*Note by translator: Sorry I don't draw the chemical structure in this paper, but I will send it by FAX if it's necessary. Please let me know.*)

(2) Physiologically active agents formulated as forms of oral administration described in the article (1) in the claim.

3. Detailed Description of the Invention

This invention relates to physiologically active agents which contain derivatives of (3-dimethylcarbamoxyphenyl) trimethyl ammonium as an active component shown in a chemical formula (I) . (*Note by translator: Sorry I don't draw the chemical structure in this paper, but I will send it by FAX if it's necessary. Please let me know.*)
(In this formula, R represents methyl sulfate radical (CH_3SO_4-) or bromine atom.)

The compound described as above (it will be described as "this compound" now on) is a known compound and has been known as an active component of an excitant of parasympathetic nerve system.
The inventor found that this compound has activities of antitumor, reducing sugar-level in blood, reducing level of lipids in blood, anti-inflammatory, antipyretic and sedative, and that it's side-effect is low even after long-term administration.

The details of this invention are described as follows;
Toxicological and pharmacological characteristics of this compound will be described afterwards.

(1) Acute toxicity

Acute toxicity of this compound has already been reported as shown in Table 1

Table 1 Acute Toxicity of This Compound

Compound tested	Animals used	Method of Administration	LD_{50} (mg/kg)
This compound (R in the formula I is Br.)	mouse	p.o.	7.5
		i.v.	0.165
	cat	p.o.	7.449
		i.v.	0.171
This compound (R in the formula I is methylsufate radical)	mouse	p.o.	12-16
		s.c.	1
		i.v.	0.3-0.4
	rabbit	s.c.	0.5-0.75
		i.v.	0.25

(2) Antitumor activity

1 x 10^6 cells of Sarcoma-180 were inoculated into hypoderm in the armpit of a JCL mouse. After 24 hours of the inoculation, 0.5 mg/kg of this compound dissolved in water was administered orally (p.o.) 10 times in every second day. Twenty five days after the inoculation, a tumor node was removed from the mouse. Inhibitory ratio of the growth (I. R. %) was calculated by the following formula.

$$(1 - T/C) \times 100 = \text{I. R. \%}$$

T: Average weight of tumor in a administered group

C: Average weight of tumor in a control group

The results are shown in Table 2.

Table 2 Antitumor Activity Against Sarcoma-180 of this Compound

Compound tested	Inhibitory Ratio of Growth (I. R. %)
This Compound (R in formula I is Br)	25.7
This Compound (R in formula I is methyl sulfate radical)	33.1

Dosage: 0.5 mg/kg x 10, administered by p.o.

(3) Reducing Activity of Blood-Sugar

The rats tested as animal models were obtained by the following procedure. Eighty mg/kg of Streptozotocin was injected into abdominal cavity of Wistar rats and after a week, positive results on their sugar levels in urine and in blood were recognized. Then insulin was administered to these rats above described and after that, reduction of sugar levels in urine and blood were found in these rats. After several days of the treatment, high levels of sugar in urine and blood were confirmed. The following experiments were carried out using these animal models.

This compound was dissolved in distilled water and 0.5 mg/kg (of rat's weight) of this compound in solution was administered orally.

Each blood sample was collected from vein in their tails and the level of glucose in the sample was determined enzymatically using RaBA kits. The results are shown in Table 3.

Table 3 Blood-Sugar Reducing Activity of this Compound

Compound tested	Reduction of blood-sugar after administration (mg/dl)	
	3 hr	6 hr
This compound (R in formula I is Br)	- 75	- 42
This compound (R in formula I is methyl sulfate radical)	- 8	- 11

Dosage: 0.5 mg/kg, by p.o.

(4) Reducing Activity of Lipids in Blood

Solid feed containing 1 % cholesterol (CR-1) was fed orally to male rabbits (Japan white variety) freely for 3 months and increase of lipid components in their sera was confirmed. These rabbits were employed as animal models with experimental arteriosclerosis. This compound was dissolved in distilled water and 0.5 mg/kg of this compound was administered to these rabbits orally. After administration, each blood sample was collected from veins in ear of the animals with the passage of time and analyzed level of lipids in the sera. Change of level of total cholesterols in blood was determined enzymatically and that of β-lipoproteins was determined with a nephelometer. The results are shown in Table 4.

Table 4 Reducing Activity of Lipids in Blood by this Compound.

Compound used	Level of β-lipoproteins (mg/dl)		Level of cholesterols (mg/dl)	
	3 hr	6 hr	3 hr	6 hr
This compound (R in formula I is Br)	- 65	- 42	- 11	- 25
This compound (R in formula I is methyl sulfate radical)	- 50	- 41	- 9	- 19
Control	+ 1	- 2	- 1	0

Dosage: 0.5 mg/kg by p.o.

(5) Anti-inflammatory Activity

1) Inhibitory Activity to Carrageenin Edema

According to the method of Van Armen et.al. (1963), 0.5 mg/kg of this compound was administered orally to 10 rats in one group compulsorily. After one hour of the administration, 0.1 ml of 1 % Carrageenin in saline was injected to sole of the foot in a right hind leg. Volume of foot was measured with the passage of time to obtain the ratio of inhibition.

2) Inhibitory Activity to Granuloma

According to the method of Winter et. al. (1963), two 30 ± 1 mg of Cotton wool pellets were planted to hypoderm in back of 6 rats in one group at right and left sides symmetrically based on its median line. After that, 0.5 mg/kg of this compound was administered orally everyday for 7 days and granuloma was removed at the 8th day. The dry weight of granuloma was determined and ratio of the inhibition was calculated.

3) Anti-Exudation Activity

According to the method of Baris et. al. (1965), a pouch was made by injection of air to hypoderm in back of 6 rats in one group and then 0.5 ml of 1 % Croton oil (in sesame oil) was injected to the pouch. 0.5 mg/kg of this compound was administered for 5 days continuously and volume of exudates was measured at the 6th day to obtain the ratio of the inhibition.

The result showed that this compound is effective as anti-inflammatory agent.

Table 5 Anti-inflammatory Activity of this Compound

Compound tested	Ratio of Inhibition (%)		
	Carrageenin Edema	Cotton pellet-Granuloma	Exudation Activity
This compound (R in formula I is Br)	19.3	15.6	21.2
This compound (R in formula I is methylsulfate radical)	9.5	20.4	23.5

Dosage: 0.5 mg/kg by P.O., Control = 0

(6) Antalgic Activity

Mechanical Stimulation Method (Stimulation Method by Pressure)

An apparatus for pressure stimulation designed by Takagi and Kameyama (Natsume Manufacturing Co. in Japan) was used for the test. An animal tested was ICR female mouse and the root of its tail was pressurized. 10 of these mice in a group showing 50 – 80 mgHg of the threshold of pain were selected.

After administration of the compound orally, its antalgic activity was decided based on time (second) required to show pseudo-escape reaction under the pressure.

Chemical Stimulation Method

According to the method of Kostet et. al. (1959), this compound was administered orally to 10 IRC female mice (5 – 6 weeks old) in a group and after 30 minutes, 0.1 ml of 0.6 % acetic acid solution per 10 grams of mouse weight was injected to abdominal cavity of the mouse. After 10 minutes, Writhing Number for 10 minutes was counted and the inhibitory ratio (%) compared to the control group was calculated using the following formula.

$$(1 - T/C) \times 100 = I.R. (\%)$$

T: Average Writhing number in the administered group.

C: Control group

The results are shown in Table 6. The results indicate that this compound is effective as an antalgic agent.

Table 6 Antalgic Activity of this Compound

Compound tested	Pseudo-escape Reaction		Inhibitory Activity (%)
	Pressure required (mmHg)	Time required (second)	
This compound (R in formula I is Br)	80	38	25.1
This compound (R in formula I is methyl sulfate radical)	78	38	20.7
Control	70	34	0

Dosage: 0.5 mg/kg, by p. o.

(7) Antipyretic Activity

According to the method of Winter et. al. (1961), suspension of 20% brewers' yeasts was injected to hypoderm of 6 rats in a group and after 10 hours fasting, this compound was administered orally. Temperature of their rectum was measured and the inhibitory ratio of pyrexia compared to that of control rats at the most effective period of the compound was calculated by the following formula.

$$\frac{C_1 - T}{C_1 - C_2} \times 100 = \text{I. R. (\%)}$$

T: Average temperature of administered group

C_1: Average temperature of pyretic control group

C_2: Average temperature of untreated control group

The results are shown in Table 7. There results indicate that this compound is effective as an antipyretic agent.

Table 7 Anti-pyretic activity of this compound

Compound tested	Ratio of Antipyretic Activity (%)
This compound (R in formula I is Br)	28.3
This compound (R in formula I is methylsulfate radical)	35.0

Dosage: 0.5 mg/kg by p.o.

The formulation of this compound will be described below.

This compound can be used as the most effective forms depending on kind of diseases and on symptoms when this compound is used as antidiabetic, antihypertensive, antitumor, antiarteriosclerosis, antiinflammatory and central nervous system control agents. This compound can also be used either as a single compound or as a mixture with diluents or other medications which are allowed to use.

This compound is administered orally or non-orally. Therefore it can be formulated optionally either for oral or for non-oral administrations.

This compound can be supplied based on its administration unit. The medication contains effective amount of this compound and it formulation can be used as forms of powder, granule, tablet, sugar-coated tablet, capsule、suppository, suspension, emulsion, ampoule and injection. As diluents, solid, liquid or semi-solid types are used. For example, the following diluents can be applied; excipients, fillers, bounding agents, moisturizers, surface active agents, emulsifiers, flavors, preservatives, liquefiers and solvents. Furthermore, either one of them or more than one of them can be used.

The physiologically active agent in this invention can be manufactured by any known processes. The active component employed in this invention can be used in the range of 0.001 % and 50 wt% in general, more preferably 0.01-5 wt %.

The physiologically active agent of this invention can be administrated either orally or non-orally to human beings and animals, more preferably orally.

Its oral administration includes sublingual administration. Its non-oral administration includes injection, for example hypodermic (subcutaneous), intramuscular, intravenous and dropping injections.

Dosage of the physiologically active agent in this invention depends on either for animals or for human beings, and on age, individual variation and/or condition of patients, and therefore there are some cases which the dosage is more or less than the range described below. But in general, its dosage for human by oral administration is 0.01-500 mg per 1 kg of weight in a day, more preferably 0.1-50 mg and its dosage by non-oral administration is 0.0001-1 mg per 1 kg of weight in a day, preferably 0.001-0.1 mg through 1 to 4 times fractional administration.

Examples are shown below. "Part" described in these examples means weight.

Example 1:

This compound (R in the formula I is Br)	1 part
Precipitated calcium carbonate	25 parts
Magnesia alumina hydrate	45 parts
Starch	30 parts

These materials are mixed uniformity and kneaded, then crushed and tableted. After drying and sieving, granules are made.

Example 2:

This compound (R in the formula I is methylsulfate radical)	0.5 part
Starch	20 parts
Lactose	55 parts
Crystalline cellulose	40 parts
Polyvinyl alcohol	3.5 parts
Water	30 parts

These materials are mixed uniformity and kneaded, then crushed and tableted. After drying and sieving, granules are made. Four parts of Calcium stearate is added to 96 parts of the dried granule and is tableted by compressing.